INVESTIGATIONS ON ALLELOPATHY IN A RED BEECH FOREST

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ABSTRACT

The possibility that allelopathy is involved in the suppression of undergrowth in a *Nothofagus fusca* forest was investigated. Potential allelochemicals in the form of conjugated phenolic compounds were leached from the foliage of *Nothofagus fusca, Pseudowintera colorata, Griselinia littoralis* and *Dicksonia lanata*. However, neither leachates nor ferulic and vanillic acids inhibited the growth of *N. fusca* seedlings in pot experiments. It is postulated that the lack of inhibition could be a result of their rapid degradation in the soil and the ability of seedlings to withstand raised phenolic levels.

INTRODUCTION

Many plants release into their environment a number of chemical compounds which inhibit the growth of other plant species. Such compounds are called allelochemicals, and the phenomenon of inhibition is termed allelopathy. Allelochemicals have been reported in a wide range of plant communities, including forests in moist climates (Whittaker and Feeny 1971). They are commonly secondary plant products such as phenolics, terpenoids, steroids, alkaloids and organic cyanides, and can be released from the plant by volatilization or leaching from leaves and stems, excretion from roots, or by the decay of dead parts. A well known example is juglone, a toxic phenolic constituent leached from the leaves of walnut trees, and responsible for the suppression of plant growth beneath the tree canopy (Grummer 1961).

Phenolics are present as flavonoids, polyphenols and simple phenols in extracts of *Nothofagus* heartwood (Hillis and Orman 1961). They are leached from the shoots of *N. truncata* by rainfall, and can inhibit the germination of white clover (K.R. Tate, pers. comm.).

Observations in a forest dominated by *Nothofagus fusca* (red beech) in the north-west Ruahine Range (about 176°10'E, 39°50'S) suggested that allelopathy could be influencing the distribution and growth of undergrowth species. Plants were markedly absent beneath the canopies of the fern, *Dicksonia lanata* and the shrubs *Pseudowintera colorata* and *Griselinia littoralis*. The ground around the base of *N. fusca* trees was often devoid of undergrowth. In addition, seedlings and saplings growing beneath a *N. fusca* canopy showed more signs of suppression than those in canopy gaps.

The possibility that phenolics acted as allelochemicals in this forest was investigated by:

1. The measurement and identification of the phenolic constituents in shoot leachates and forest soil.
2. Determining the effects of litter, shoot leachates and phenolic compounds on the growth of *N. fusca* seedlings.

METHODS

IDENTIFICATION OF PHENOLICS

Twigs of *N. fusca* and *P. colorata* were collected from the forest in summer and soaked in distilled water for 4 days. The resulting leachate was hydrolysed by refluxing in 1M HCl for two hours. Phenolics are usually present in plant leachates in conjugated forms, and hydrolysis is necessary to release the phenolic aglycones for identification. The hydrolysate was then extracted with ether and the ether phase dried and made up in methanol. This methanolic solution was applied to two-dimensional, thin layer chromatography on silica gel. The first solvent was the non-aqueous phase of a 10:7:3 (v/v) benzene-acetic acid-water mixture, and the second was 2% (v/v) acetic acid. After drying, the chromatogram was fumed with NH_3, then observed under short wave ultraviolet light. Chromatograms were also observed under normal light after spraying first with diazotized *p*-nitroaniline, followed by 30% NaOH.

TOTAL PHENOLIC CONCENTRATION OF LEACHATES AND SOIL

The total concentration of phenolics was measured using Folin-Ciocalteu reagent and the method of Swain and Hillis (1959). Shoot leachates of *N. fusca, P. colorata, G. littoralis,* and *D. lanata,* prepared as previously described, were measured before and after hydrolysis. Aqueous soil extracts were obtained by mixing 50 ml of distilled water with 50 ml of soil and leaving for 48 hours.

EFFECTS OF PHENOLICS ON THE GROWTH OF *N. FUSCA* SEEDLINGS

Seedlings between one and two years of age were collected from the forest in spring and grown in a fertilized peat-sand mix in a glasshouse. Pots were watered from below by capillary feeding, and additional water was applied to the top as required and following leachate application. The seedlings were grown for 58 days. Initially the heights of all seedlings and the total dry weights of a harvested subsample were measured. At the final measurement the heights and total dry weights of all seedlings were measured. There were 5 seedlings for each treatment and they were grown singly in pots.

The following treatments were made:

1. Addition of litter
   10 g of fresh shoots of *P. colorata* and *N. fusca* were macerated and incorporated into the surface soil of the pot.

2. Application of leachate
   10 ml of each leachate were applied to the surface of the pot at fortnightly intervals (4 applications).

3. Application of phenolic solution
   Solutions containing 200 ppm of ferulic acid or vanillic acid, phenolics commonly found to be allelopathic, were applied in a similar manner to the leachates. Each addition contained 2 mg of each phenolic acid.

RESULTS

The chromatograms showed 11 spots for the *N. fusca* leachate and 10 for the *P. colorata* leachate. These spots represent simple phenolic acids found in the leachate hydrolysate. Using the details given by Ibrahim and Towers (1960) for a number of common phenolic acids, three spots were tentatively identified. These were, for *N. fusca*, gentisic acid, and for *P. colorata*, caffeic acid and a *cis-trans* isomers of sinapic acid.

The foliage leachates contained conjugated phenolic compounds. Total phenolic concentrations of plant and soil extracts are shown in Table 1. The range of concentration detected was 1 ppm to 300 ppm.

TABLE 1. TOTAL PHENOLIC CONCENTRATIONS IN PLANT AND SOIL EXTRACTS.

Extract	Phenolic Content (ppm)
N. fusca shoots	99*
P. colorata shoots	65*
G. littoralis shoots	15
D. lanata fronds	257
Rotting *N. fusca* wood (from forest floor)	10.3*
Mineral soil	2.5*

*Average of two samples collected in different months.

Soil extracts from the pot experiment showed phenolic concentrations ranging from 1.9 to 2.5 ppm. The concentrations were not altered by the addition of solutions containing phenolics.

None of the treatments had a significant effect on seedling growth (Table 2) and no differences in the appearance of the seedlings were noted.

DISCUSSION

Considerable quantities of phenolic compounds are leached from the foliage of four of the common species in a *Nothofagus fusca* forest, yet only low phenolic levels are found in the forest soil. This means that phenolics entering the soil via rainfall must be rapidly removed from the soil soluble form as a result of microbial degradation, complexing with humic materials (Müller and Chou 1972), or leaching from the soil. Because soil phenolic levels are relatively low it seems unlikely that phenolics could act as allelochemicals. Soil phenolic levels of the magnitude reported here have been observed in a number of soils (Whitehead 1964).

TABLE 2. FINAL HEIGHT AND DRY WEIGHT MEASUREMENTS OF SEEDLINGS. For each treatment N = 5.

	Height (mm) ± S.D.	Dry weight (mg) ± S.D.
Litter Treatments		
Control	246.8 ± 65.6	1054.3 ± 441.5
P. colorata litter	240.4 ± 72.2	711.3 ± 362.7
N. fusca litter	190.0 ± 28.8	975.0 ± 444.8
Leachate Treatments		
Control	102.0 ± 29.1	225.8 ± 84.5
N. fusca	105.2 ± 18.0	268.8 ± 55.6
P. colorata	123.6 ± 29.1	312.0 ± 113.6
D. lanata	95.2 ± 25.2	253.0 ± 138.9
G. littoralis	114.0 ± 28.2	237.1 ± 91.7
Ferulic acid	110.4 ± 35.4	206.8 ± 81.5
Vanillic acid	112.8 ± 26.7	284.8 ± 149.1

The ineffectiveness of allelopathy is further demonstrated by the preference of *N. fusca* seedlings for rotting wood growing sites and in the results of the growth experiment. Rotting wood has four times the total phenolic content of the mineral soil, but is more favourable for seedling growth and survival (June and Ogden 1975). The growth experiment also demonstrated the apparent immunity of the seedlings to phenolic solutions with known toxic properties. However, this experiment was not carried out under the growth limiting conditions found in the forest. If the allelochemicals such as phenolic acids (Glass 1973), may act by inhibiting nutrient uptake, then the inhibition would only be effective if the nutrient supply is sub-optimal (as in a forest). Ideally, the experiment should be carried out under conditions similar to those in the forest environment. It is difficult to know if, under the experimental conditions used, the abundant nutrient supply may have overcome the allelopathic effects on nutrient uptake.

Allelopathy in a forest community would be a complex interaction, with many species contributing potential allelochemicals, and with a number of other environmental factors influencing seedling growth. Some of these other factors are clearly involved in the suppression of undergrowth. The low levels of light intensity as a result of shading by tree, shrub and fern canopies can inhibit growth (June and Ogden 1975). Light intensities beneath the *Dicksonia lanata* and *Pseudowintera colorata* canopies are below the compensation point for *Nothofagus fusca* seedlings. Seedling establishment also may be prevented by a dense litter layer. This is particularly noticeable beneath *D. lanata*, where the seedlings germinate in the litter and their roots fail to penetrate to the mineral soil before they succumb to drought. Root competition from mature trees could account for the suppression of seedlings and saplings, but no evidence is available to support this.

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